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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

088.000426

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)

N/A

09/720549

INTERNATIONAL APPLICATION NO.

PCT/EP99/04385

INTERNATIONAL FILING DATE

23 June 1999

PRIORITY DATE CLAIMED

24 June 1998

TITLE OF INVENTION

APPARATUS AND METHOD FOR INTERFERING WITH PATHOLOGICAL CELLS SURVIVAL PROCESSES

APPLICANT(S) FOR DO/EO/US

Santi TOFANI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☒ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☒ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☐ Other items or information:

Certificate of Mailing by Express Mail Label No. EL692207209 US

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.53) 09/7720549	INTERNATIONAL APPLICATION NO. PCT/EP99/04385	ATTORNEY'S DOCKET NUMBER 088.000426
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21. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

- ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **\$1,000.00**
- ☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **\$860.00**
- ☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **\$710.00**
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **\$690.00**
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **\$100.00**

CALCULATIONS PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =				\$860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	56 - 20 =	36	x \$18.00	\$648.00	
Independent claims	5 - 3 =	2	x \$80.00	\$160.00	
Multiple Dependent Claims (check if applicable). <input type="checkbox"/>				\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$1,668.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). <input checked="" type="checkbox"/>				\$834.00	
SUBTOTAL =				\$834.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				\$0.00	
TOTAL NATIONAL FEE =				\$834.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/>				\$0.00	
TOTAL FEES ENCLOSED =				\$834.00	
				Amount to be: refunded	\$
				charged	\$

- ☒ A check in the amount of **\$834.00** to cover the above fees is enclosed.
- ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.
- ☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **50-0822** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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SIGNATURE

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NAME

33,034

REGISTRATION NUMBER

December 22, 2000

DATE

**STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) & 1.27(b))--INDEPENDENT INVENTOR**

Docket Number (Optional)

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS, SEND TO: Assistant Commissioner for Patents, Washington, DC 20231

09/720549

528 Rec'd PCT/PTO 22 DEC 2000

U.S. National Stage Patent Application
Attorney Docket No. 088.000426

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re: the Matter of:

U.S. National Stage Patent Application

Examiner: N/A

Applicant: Santi TOFANI

Art Unit: N/A

For: APPARATUS AND METHOD FOR INTERFERING WITH PATHOLOGICAL CELLS
SURVIVAL PROCESSES

Filed: December 22, 2000

CERTIFICATE OF MAILING BY EXPRESS MAIL

I certify that this Preliminary Amendment is being deposited on December 22, 2000 with the U.S. Postal Service "Express Mail Post Office Addressee" service under 37 C.F.R. §1.10 and is addressed to The Assistant Commissioner for Patents, Washington, D.C. 20231.


Robert P. Simpson

Express Mail #EL692207209 US

PRELIMINARY AMENDMENT

BOX PCT
Commissioner for Patents
Washington, DC 20231

Honorable Sir:

This Preliminary Amendment is submitted along with the above-identified application and eliminates multiple dependent claims. This Amendment contains no new matter.

23. Apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo, comprising:

means for generating electromagnetic extremely low frequency (ELF) fields over said working environment;

means for modulating said ELF fields associated to said means for generating, said means for modulating said ELF fields setting said ELF fields as recited in a predetermined function of amplitude of intensity between 1 and 100 mT and frequency between 1 and 1000 Hz versus time.

24. Apparatus as recited in Claim 21 wherein said means for modulating said S fields comprises program means that set said intensity following a plurality of predetermined step values I_{S1} , I_{S2} , I_{S3} , I_{Sn} for corresponding time intervals T_1 , T_2 , T_3 , T_n .

25. Apparatus as recited in Claim 22 wherein said means for modulating said S fields comprises program means that set said intensity following a plurality of predetermined step values I_{S1} , I_{S2} , I_{S3} , I_{Sn} for corresponding time intervals T_1 , T_2 , T_3 , T_n .

26. Apparatus as recited in Claim 21 wherein said means for modulating said ELF fields comprises program means that set said intensity amplitude following a plurality of predetermined step values I_{ELF1} , I_{ELF2} , I_{ELF3} , I_{ELFn} for corresponding time intervals T_1 , T_2 , T_3 , T_n .

27. Apparatus as recited in Claim 23 wherein said means for modulating said ELF fields comprises program means that set said intensity amplitude following a plurality of predetermined step values I_{ELF1} , I_{ELF2} , I_{ELF3} , I_{ELFn} for corresponding time intervals T_1 , T_2 , T_3 , T_n .

28. Apparatus as recited in Claim 21 wherein said means for modulating said ELF fields comprises program means that set said frequency following a plurality of predetermined step values f_1, f_2, f_3, f_n , for corresponding time intervals T_1, T_2, T_3, T_n , said step values being comprised between 10 and 100 Hz.

29. Apparatus as recited in Claim 23 wherein said means for modulating said ELF fields comprises program means that set said frequency following a plurality of predetermined step values f_1, f_2, f_3, f_n , for corresponding time intervals T_1, T_2, T_3, T_n , said step values being comprised between 10 and 100 Hz.

30. Apparatus as recited in Claim 21, wherein said means for modulating said S and ELF fields comprises program means that set an S/ELF ratio as recited in a plurality of predetermined step values $IS_1/IELF_1, IS_2/IELF_2, IS_3/IELF_3, IS_n/IELF_n$, for corresponding time intervals T_1, T_2, T_3, T_n .

31. Apparatus as recited in Claim 30, wherein said program means set said S and ELF fields as recited in an overall intensity between 1 and 30 mT and respectively a ratio S/ELF comprised between 0,1 and 10.

32. Apparatus as recited in Claim 30, wherein said program means set said S and ELF fields as recited in an overall intensity between 1 and 10 mT and respectively a ratio S/ELF comprised between 0,5 and 5.

33. Apparatus as recited in Claim 24 wherein said program means set said time intervals between 1 and 40 minutes.

34. Apparatus as recited in Claim 25 wherein said program means set said time intervals between 1 and 40 minutes.

35. Apparatus as recited in Claim 26 wherein said program means set said time intervals between 1 and 40 minutes.

36. Apparatus as recited in Claim 27 wherein said program means set said time intervals between 1 and 40 minutes.

37. Apparatus as recited in Claim 28 wherein said program means set said time intervals between 1 and 40 minutes.

38. Apparatus as recited in Claim 29 wherein said program means set said time intervals between 1 and 40 minutes.

39. Apparatus as recited in Claim 30 wherein said program means set said time intervals between 1 and 40 minutes.

40. Apparatus as recited in Claim 31 wherein said program means set said time intervals between 1 and 40 minutes.

41. Apparatus as recited in Claim 32 wherein said program means set said time intervals between 1 and 40 minutes.

42. Apparatus as recited in Claim 1 wherein at least a portion of said working environment is defined by walls permeable to said fields.

43. Apparatus as recited in Claim 2 wherein at least a portion of said working environment is defined by walls permeable to said fields.

44. Apparatus as recited in Claim 3 wherein at least a portion of said working environment is defined by walls permeable to said fields.

45. Apparatus as recited in Claim 1 wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil respectively surrounding at least a portion of said working environment, said means for modulating providing to said coils DC and/or AC current respectively.

46. Apparatus as recited in Claim 2 wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil respectively surrounding at least a portion of said working environment, said means for modulating providing to said coils DC and/or AC current respectively.

47. Apparatus as recited in Claim 3 wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil respectively surrounding at least a portion of said working environment, said means for modulating providing to said coils DC and/or AC current respectively.

48. Apparatus as recited in Claim 1 wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil coaxial to each other, said working environment being placed between said first and a second coil and said means for modulating providing to said coils DC and/or AC current respectively.

49. Apparatus as recited in Claim 2 wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil coaxial to each other, said working environment being placed between said first and a second coil and said means for modulating providing to said coils DC and/or AC current respectively.

50. Apparatus as recited in Claim 3 wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil coaxial to each other, said working environment being placed between said first and a second coil and said means for modulating providing to said coils DC and/or AC current respectively.

51. Apparatus as recited in Claim 1 wherein means are provided for creating through said working environment a static electric field, or a low frequency variable electric field up to 1000 Hz, having intensity up to 20 kV/m.

52. Apparatus as recited in Claim 2 wherein means are provided for creating through said working environment a static electric field, or a low frequency variable electric field up to 1000 Hz, having intensity up to 20 kV/m.

53. Apparatus as recited in Claim 3 wherein means are provided for creating through said working environment a static electric field, or a low frequency variable electric field up to 1000 Hz, having intensity up to 20 kV/m.

54. A method of using SELF non-thermal fields for selectively interfering with pathological cells' survival, such as in particular cells affected by cancer, viral infections, autoimmune diseases, neurodegenerative disorders and AIDS comprising applying said SELF non-thermal fields having intensity in the range of between 1 and 100 mT.

55. A method of using SELF non-thermal fields as recited in Claim 54 wherein said method comprises applying S fields followed by ELF fields.

56. A method of using SELF non-thermal fields as recited in Claim 54 wherein said method comprises applying ELF fields followed by S fields.

57. A method of using SELF non-thermal fields as recited in Claim 54 wherein said method comprises applying ELF and S fields concurrently.

58. A method of using SELF non-thermal fields as recited in Claim 54 wherein said method comprises applying S fields alone.

59. A method of using SELF non-thermal fields as recited in Claim 54 wherein said method comprises applying ELF fields alone.

60. A method of using SELF non-thermal fields as recited in Claim 54 wherein said ELF fields have a field frequency in the range of between 1 and 1000 Hz.

61. A method of using SELF non-thermal fields for biotechnological genes modifications, comprising applying said SELF non-thermal fields to said biotechnological genes to be modified, where said SELF non-thermal fields have intensity in the range between 1 and 100 mT.

62. A method of using SELF non-thermal fields as recited in Claim 61 wherein said method comprises applying S fields followed by ELF fields.

63. A method of using SELF non-thermal fields as recited in Claim 61 wherein said method comprises applying ELF fields followed by S fields.

64. A method of using SELF non-thermal fields as recited in Claim 61 wherein said method comprises applying ELF and S fields concurrently.

65. A method of using SELF non-thermal fields as recited in Claim 61 wherein said method comprises applying S fields alone.

66. A method of using SELF non-thermal fields as recited in Claim 61 wherein said method comprises applying ELF fields alone.

67. A method of using SELF non-thermal fields as recited in Claim 61 wherein said ELF fields have a field frequency in the range of between 1 and 1000 Hz.

68. A method of using SELF non-thermal fields as recited in Claim 61 wherein biotechnological gene is a mutant p53 gene.

69. A method of using SELF non-thermal fields as recited in Claim 54, further including the step of applying chemical substances in addition to the SELF fields.

70. A method of using SELF non-thermal fields as recited in Claim 61, further including the step of applying chemical substances in addition to the SELF fields.

71. A method of using SELF non-thermal fields as recited in Claim 54, wherein said SELF non-thermal fields are applied in different sequences, and said sequences are set for time intervals T_1 , T_2 , T_3 , T_n , and wherein in said time intervals the intensity of said S and/or ELF fields are set at steady values Is_1 , Is_2 , Is_3 , Is_n ; $Ielf_1$, $Ielf_2$, $Ielf_3$, $Ielf_n$, $Is_1/Ielf_1$, $Is_2/Ielf_2$, $Is_3/Ielf_3$, $Is_n/Ielf_n$, respectively.

72. A method of using SELF non-thermal fields as recited in Claim 61, wherein said SELF non-thermal fields are applied in different sequences, and said sequences are set for time intervals T_1 , T_2 , T_3 , T_n , and wherein in said time intervals the intensity of said S and/or ELF fields are set at steady values Is_1 , Is_2 , Is_3 , Is_n ; $Ielf_1$, $Ielf_2$, $Ielf_3$, $Ielf_n$, $Is_1/Ielf_1$, $Is_2/Ielf_2$, $Is_3/Ielf_3$, $Is_n/Ielf_n$, respectively.

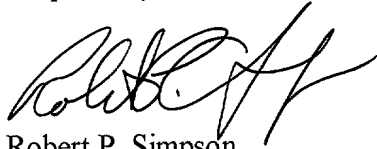
73. A method of using SELF non-thermal fields as recited in Claim 54, wherein said S and ELF fields are set at an overall intensity in the range of between 1 and 30 mT with a S/ELF ratio in the range of between 0.1 and 10.

74. A method of using SELF non-thermal fields as recited in Claim 61, wherein said S and ELF fields are set at an overall intensity in the range of between 1 and 30 mT with a S/ELF ratio in the range of between 0.1 and 10.

75. A method of using SELF non-thermal fields as recited in Claim 54, wherein said S and ELF fields are set at an overall intensity in a range between 1 and 10 mT with a S/ELF ratio in the range of between 0.5 and 2.5.

76. A method of using SELF non-thermal fields as recited in Claim 61, wherein said S and ELF fields are set at an overall intensity in a range between 1 and 10 mT with a S/ELF ratio in the range of between 0.5 and 2.5.

Respectfully submitted,



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RPS/
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TITLE

APPARATUS AND METHOD FOR INTERFERING WITH PATHOLOGICAL
CELLS SURVIVAL PROCESSES

DESCRIPTION

5 Field of the invention

The present invention generally relates to an apparatus for interfering with pathological cells survival processes.

In addition, the invention relates to a
10 microbiological method carried out by such apparatus for
interfering with pathological cells survival, in
particular cells affected by cancer and other diseases
caused by alterations in the mechanism of cell survival.

In particular, the interference is induced by means
15 of static (S) and extremely low frequency electromagnetic
(ELF) fields produced by the apparatus.

Magnetic Static fields and Extremely Low Frequency electromagnetic fields are hereinafter referred to also as S and ELF, respectively. Moreover, any possible combination of different sequences of S and/or ELF fields, such as S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, will hereinafter be referred to also as SELF fields.

25 Background of the invention

It is known that pericellular fields and currents induced by an Extremely Low Frequency (ELF) electromagnetic field, whose frequency range is from 1 Hz to 300 Hz and perhaps up to 1000 Hz, induce within the cell certain membrane electrochemical events which are important for primary biologic signal transduction and amplification processes.

These biochemically mediated events then produce cytoplasmic second messengers and internal effectors such as free Ca^{++} and protein phosphorylases (kinases) which in

turn trigger certain changes in the biosynthesis of macromolecules as well as bring about alterations in cellular growth differentiation and functional properties [1M. Blank, 1993].

5 Further, the possibility that S and ELF fields affect the DNA synthesis, DNA integrity, transcription and translation has been documented [2Liboff 1984, 3Tofani 1995, 4Goodman 1991, 5Phillips 1992].

10 A possible physical mechanism to account for some of the experimental findings is the direct effect on ions (i.e. Ca^{++}) or on ligand binding at the cell membrane [6Liboff 1985, 7Chiabrera 1985, 8Lednev 1991, 9Blanchard 1994].

15 The possibility of influencing variations of Ca^{++} metabolism may lead to cell apoptosis (programmed cell death) [10Preston, 11Trump 1997].

20 Another physical interaction mechanism is related to the possibility of influencing the kinetics of appropriate cell signalling pathways of the cell (including calcium metabolism) through a field direct effect on electron-spin motion of atoms and molecules with unpaired electrons. This influencing may affect the recombination ratio of a spin correlated free radical pair and consequently on redox signalling [12Grundler 1992; 25 13Polk 1992; 14Walleczek and Budinger 1992; 15Adey 1993].

In particular, the spin singlet-triplet energetic level transition in a free radical is critical for increasing the recombination ratio of spin correlated free radical pairs.

30 The possibility for low level, non thermal (with intensity up to 30 mT) S and ELF magnetic fields to influence in vitro the kinetics and efficacy of radical pair reactions is known from magnetochemistry [16Steiner 1989].

35 Naturally occurring free radicals have an oxygen-

or nitrogen-based unpaired electron such as superoxide anion, hydroxyl radical and nitric oxide. These Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) can target proteins providing an obvious mechanistic explanation for free radicals-mediated signalling events. These events may influence growth factors, ion transport (i.e. Ca^{++} channels), transcription, apoptosis [17Lander 1997].

Apoptosis is a morphologically distinct form of programmed cell death that is connected in cell survival processes playing an important role during development, homeostasis, and in many diseases including cancer, acquired immunodeficiency syndrome, and neurodegenerative disorders, as well as in other diseases that similarly to those are characterised by altered cell survival processes. Apoptosis occurs through the activation of a cell-intrinsic suicide program. The basic genetic mechanism of apoptosis appears to be present in essentially all mammalian cells at all times, but the activation of this suicide program is regulated by many different signals that originate from both the intracellular and the extracellular environment.

Among all the genes involved in apoptosis regulation, the p53 gene is receiving much attention. This gene, which encodes a transcription factor and is common in many human cancers, mediates the cellular responses to some environmental damage. The p53 protein either can temporarily stop cell division, so that the cell can repair altered DNA, or can pilot the cell to an apoptotic death.

Published data support that p53 appears in apoptosis through a three step process: 1) transcriptional induction of redox-related genes: 2) the formation of reactive oxygen species and 3) the oxidative degradation of mitochondria components, culminating in cell death

[¹⁸Polyak 1997] .

In addition anti-oxidative agents are combined with drugs in the treatment of hypoxia tumour cells ¹⁹ [Walch, 1988] and in the influence of vascular growth factor
5 ²⁰[Amirkhosravi, 1998].

Moreover, published data are supporting the idea that pathological cells answer differently than normal cells to ELF fields stimuli. According to ²¹Cadossi [1992], lymphocytes from normal patients respond differently than
10 lymphocytes from Down's syndrome, AIDS and chronic lymphocytic leukaemia patients when exposed to ELF fields (previously with mitogen).

It is also recognised that Ca^{++} influx across the membrane is influenced by ELF fields in leukaemic
15 lymphocytes but not in normal lymphocytes [²²Walleczek, 1996].

Altered cell survival processes come with electric disorders and different electrical behavior. In fact, rapidly proliferating and transformed cells have
20 electrically depolarized cell membranes if compared with normal cells [²³Binggeli, 1986; ²⁴ Marino 1994]. It has also been shown that epithelial cells lose their transepithelial potential during carcinogenesis [²⁵Davies 1987; ²⁶ Goller 1986; ²⁷ Capko, 1996]. This different
25 electrical behavior of tumor cells compared with normal cells is the basis for a newly proposed cancer diagnostic modality [²⁸Cuzick 1998]. In addition, the concentration of free radicals in transformed cells and tissues is higher than in non-transformed ones [²⁹Szatrowski 1991; ³⁰
30 Shulyakovskaya 1993; ³¹ Iwagaki 1995].

With reference to chemotherapy all efforts are devoted to the target of inducing cell apoptosis in vivo instead of killing them, through Signal Transduction Directed Therapy (STDT) of cancer [³²Levin, 1998].

35 Signal Transduction is a functional term that

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as in DE 4122380A1 and US 5156587. However, these documents describe the use of sinusoidal fields only at a fixed net frequency and at a fixed intensity, with the possibility to sweep only a limited range of energy levels
5 inside the cellular tissue.

Summary of the invention

It is an object of the present invention to provide a method for interfering with cell survival processes (i.e. inducing apoptosis) of living pathological cells
10 (i.e. cancer cells) by using magnetic fields without adversely affecting normal cells.

It is another object of the invention to provide an apparatus for interfering with pathological cells survival processes.

15 The former and other objects are reached by the method for interfering with pathological cells survival according to the invention whose characteristic is to apply to living pathological cells (i.e. cancer cells and cells affected by other diseases caused by alterations in
20 the mechanism of cell survival) non thermal SELF magnetic fields to induce apoptosis in a selective way.

For the purposes of the invention SELF fields are to be considered as different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields
25 followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone.

The concept underlying the method according to the invention is that SELF fields interfere with cell signalling sustaining cell pathological behaviour inside
30 pathological cells, i.e. on redox signalling through free radicals, thus restoring the cell survival processes, i.e. inducing directly or indirectly apoptosis through a modification of p53 gene expression.

This method is supposed to recombine oxygen-based
35 free radicals and may also be used as an anti-oxidative

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agent. It's combination with drugs in the treatment of hypoxia tumour cells and in the influence of vascular growth factor may also be considered.

The reason why SELF fields selectively induce apoptosis in pathological cells (i.e. cancer cells) may be related to the altered electrical behaviour of pathological cells compared with that of normal cells.

For these reasons SELF fields can induce directly or indirectly a signal programmed cell death (apoptosis), in vitro and in vivo, without causing any adverse effect.

In the hypothesis that free radicals recombination is at the basis of the expected biological effects on pathological cells (i.e., anti-tumour activity) the transition between singlet-triplet of unpaired electron in oxygen based free radicals has to be considered. In fact this transition, which depends on the applied magnetic field, is critical for increasing the recombination ratio of a spin correlated free radical pair. However, the reaction centres related to the expected anti tumor effect are unknown and therefore the lifetime of the spin states and the energy splitting between singlet and triplet states cannot be precisely determined from the spin hamiltonian [³⁷Haberkorn 1979, ³⁸ Lersch 1983].

To encompass this problem, according to the invention, sequences of S magnetic fields with different intensity modulated in amplitude can be used, with the superimposition of ELF magnetic fields. The use of modulated fields is in agreement with the need for reaching optimal condition(s) for the singlet-triplet spin state conversion required for the free radical recombination processes [¹³Polk 1992].

For these reasons, S, ELF or SELF fields have higher probability to induce the expected biological effects if they are modulated following a predetermined function of intensity and or frequency versus time, since this way the

probability to induce the above transition is higher.

The different sequences of S and/or ELF fields sequences are advantageously set for time intervals T_1 , T_2 , \dots , T_n , wherein the intensity I_s , I_{ELF} and their ratio I_s/I_{ELF} are
5 set at steady values I_{s1} , I_{s2} , \dots , I_{sn} ; I_{ELF1} , I_{ELF2} , \dots , I_{ELFn} , I_{s1}/I_{ELF1} , I_{s2}/I_{ELF2} , \dots , I_{sn}/I_{ELFn} , respectively.

For the same reasons modulated SELF non thermal fields can be potentially used for treatment of cells affected by many diseases like viral infections, AIDS,
10 autoimmune diseases, etc., where the alteration of cell survival contributes to their pathogenesis.

According to another aspect of the invention, an apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo has the
15 characteristic of comprising means for generating static magnetic (S) fields crossing a working environment and means for generating electromagnetic extremely low frequency (ELF) fields in the working environment alone or in addition to the S fields.

20 Means are provided for modulating the S fields associated to the means for generating S fields and varying the intensity of the S fields between 1 and 100 mT and preferably from 1 to 30 mT.

Means are also provided for modulating the ELF
25 fields alone or associated to the S fields at a frequency between 1 and 1000 Hz with intensity comprised between 1 and 30 mT. Preferably the ELF fields have a frequency between 10 and 100 Hz.

In a particular embodiment of the invention the
30 means for modulating the S fields comprises program means that alternatively or in combination:

- set the intensity following a plurality of predetermined step values I_{s1} , I_{s2} , \dots , I_{sn} for corresponding time intervals T_1 , T_2 , \dots , T_n ;
- 35 - set the intensity amplitude following a plurality of

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predetermined step values I_{ELF1} , I_{ELF2} , ..., I_{ELFn} for corresponding time intervals T_1 , T_2 , ..., T_n ;

- set the frequency following a plurality of predetermined step values f_1 , f_2 , ..., f_n , for corresponding
5 time intervals T_1 , T_2 , ..., T_n ;

- set an S/ELF ratio according to a plurality of predetermined step values I_{S1}/I_{ELF1} , I_{S2}/I_{ELF2} , ..., I_{Sn}/I_{ELFn} , for corresponding time intervals T_1 , T_2 , ..., T_n .

Preferably, the program means set the S and ELF
10 fields according to an overall intensity between 1 and 30 mT and respectively a ratio S/ELF comprised between 0,1 and 10 and, in a particularly preferred embodiment, according to an overall intensity between 1 and 10 mT and respectively a ratio S/ELF comprised between 0,5 and 5.

15 The time intervals are preferably set between 1 and 40 minutes.

At least a portion of the working environment is defined by walls permeable to the S and ELF fields. At least a portion of the working environment is also
20 advantageously adjacent to a first and a second coil respectively and the means for modulating supplying to the coils DC and AC current respectively.

Brief description of the drawings

Several embodiments of the apparatus are shown in
25 the attached drawings, given as an example and not limitative, wherein:

- Figure 1 shows a diagrammatical view of a first embodiment of an apparatus according to the invention;
- Figures 2 to 4 show block diagrams of a second third
30 and fourth embodiment of an apparatus according to the invention, respectively;
- Figure 5A shows a diagrammatic function of field intensity versus time, as programmable in the apparatus according to the invention;
- 35 - Figure 5B shows a diagrammatic function of field

- Figure 5C shows a diagrammatic function of field intensity and frequency versus time.

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cells survival both in vitro and in vivo has two Helmholtz coils 43 and 44 located coaxial to each other at the opposite sides of the working environment 41. An amplifier 46 is used between the modulator 45 and the coils 43 and 44, through a shunt element 47, which is also connected to a personal computer 49.

Each apparatus can be used for producing SELF modulated non thermal fields for interfering with pathological cells survival.

With reference to figures 5A to 5C, an example of the programming of the apparatus is given wherein the modulation of intensity, frequency and intensity ratio between S and ELF fields is carried out.

In figure 5A the way in which the intensity I may vary versus time. I_1, I_2, I_3, I_n are the intensity or field strength (mT) of either the S field, or of the ELF field, or the overall intensity $I_s + I_{ELF}$.

In figure 5B, when both fields S and ELF are present, it is possible to modulate not only their intensity or intensity amplitude, but also their ratio I_s/I_{ELF} . For example, different ratios 1; 1.5; 2; etc. can be used for time intervals $T_1, T_2; T_3$; etc.

Also the frequency can be modulated as shown in figure 5C. The frequency may also be modulated in two or more following intervals T_1, T_2 , wherein the same intensity I_{1-2} is applied.

Starting from the basic examples of figures 5A-5C a sequence of modulated S, ELF, S+ELF fields can be produced that can also be repeated cyclically.

The method according to the invention will now be described in more detail by way of specific examples.

EXAMPLE 1

In this experiment the capability of inducing apoptosis by SELF magnetic field as a function of field intensity and frequency was studied in vitro.

Human colon adenocarcinoma cell line (WiDr) grown in confluent monolayers in T25 flasks was used for the experiment. For each exposure condition 6 flasks containing each about 10 millions cells were used, 3 exposed and 3 shame-exposed (i.e. not exposed).

During the exposure the flasks were held between two coils connected with a circuit providing DC and AC currents up to 100 Hertz. The temperature was continuously monitored and maintained at $37 \pm 0,2$ °C.

The exposure duration was 20 minutes for each experiment and the SELF field was maintained constant. After 3 hours the cells were treated with May- Grunwald-Giemsa. Apoptosis was assessed by counting the number of apoptotic nuclei per 10 high power fields (HPF) by using an optic microscope.

The amount of induced apoptosis was evaluated by the ratio between the number of apoptotic cells found in the exposure group and the number of apoptotic cells found in the shame-exposed group, that is the group not exposed to the magnetic fields according to the invention.

Table 1 reports the results obtained in different exposure conditions.

TABLE 1

exposure conditions	SELF field composition	frequency (Hz)	field intensity (Static + ELF rms) mT	apoptosis ratio
A	S (static)	-	(0.5 + 0)	1
B	S	-	(1 + 0)	1
C	S	-	(2 + 0)	1.2
D	S	-	(3 + 0)	2
E	S	-	(4 + 0)	2,3
F	S	-	(10 + 0)	2.2
G	S	-	(20 + 0)	2.2
H	S	-	(30 + 0)	2.3
I	ELF	16	(0 + 3)	2.2

L	ELF	33	(0 + 3)	2.2
M	ELF	50	(0 + 3)	2.1
N	ELF	50	(0 + 7)	2,1
O	ELF	66	(0 + 3)	2.2
P	ELF	83	(0 + 3)	2.3
Q	ELF	100	(0 + 3)	2.1
R	S + ELF	50	(4 + 3)	2.1
S	S + ELF	50	50% of time (3 + 1) 50% of time (4,5 + 1,5)	2.2

All the results were statistically highly significant (at the t Student test). From Table 1 we can see that the apoptosis effect appears at 2 mT and doubles starting from 3 mT.

Another important finding is that apoptosis doesn't depend upon SELF field frequency. In other words during the lifetime of the mechanism operating the biological effect (apoptosis) the ELF field is seen as essentially constant. This means that between the two hypothesised mechanism, free- radicals (occurring in a time scale of nano- to microsecond) and ion resonance-like mechanisms, the free radical one is playing the role [³⁹Scaiano, 1994, ⁴⁰Engstrom, 1997].

EXAMPLE 2

In this experiment the selective effect of SELF magnetic fields was verified exposing three cell lines. Two lines were malignant, human colon adenocarcinoma cells (WiDr) and human breast cancer cells (MCF-7). The normal cell line was human lung fibroblast (MRC-5).

As in the example 1 each cell line was grown in confluent monolayers in T25 flasks. The experimental protocol was the same as in example 1. Six flasks (3 exposed and three shame-exposed) for each cell line were exposed for 20 minutes. Apoptosis was evaluated after 3 hours. The exposure conditions used were the R type of Table 1.

The results are reported in Table 2.

TABLE 2

cell line	apoptosis ratio
WiDr	2.1
MCF-7	1.4
MRC-5	1

As shown in Table 2 only cancer cells reported an apoptosis increment statistically highly significant, whereas the normal cell line didn't. The difference in percentage of apoptosis between the two cancer cell lines was expected due to the two different duplication times. In fact WiDr duplicates faster than MCF-7. The results were evaluated at t Student test.

EXAMPLE 3

In this example nude mice (nu/nu) bearing subcutaneous tumour masses were used to assess the influence of SELF magnetic fields on tumour growth inhibition.

Each mouse was inoculated subcutaneously with 10 million human colon adenocarcinoma cells (WiDr). Two experiments were successively carried out.

In the first experiment, 36 female mice were randomly assigned to 4 experimental groups, each formed by 6 exposed and 3 shame-exposed for a total of 24 animals exposed to 4 different SELF magnetic fields and 12 shame-exposed.

A Static Electric Field up to 6 kV/m was also applied to eventually take advantage of the different electrical behaviour between tumoral and normal tissues [41Thornton, 1984; 42Barsamian, 1987]

In the second experiment 24 female mice were randomly assigned to 2 experimental groups, formed by 12 exposed to the SELF exposure condition which gave the best results among the four exposure conditions used in the previous

experiment (exposure condition number 4), and 12 shame-exposed.

All the mice of both experiments were divided into experimental groups after the tumor masses for each animal were palpable.

The animals were exposed for 70 minutes, once a day, for 5 days a week, for 4 weeks. During the exposure each mouse was put in a single box made of Plexiglas held between two coils connected to a circuit providing DC and AC current up to 100 Hz respectively.

Nude mice were kept under specific pathogen free conditions and supplied with "ad libitum" diet. All the tests were conducted in accordance with the protocol issued by N.I.H. (US National Institute of Health) and N.C.I. (US National Cancer Institute).

The tumor masses were measured twice a week and their volume calculated in mm^3 according to the formula:

$$[(\text{major diameter}) \times (\text{minor diameter squared})] / 2.$$

After 4 weeks the animals were sacrificed and autopsied. Tumor masses were extracted, weighed and measured. Portions of tumors were used for different analysis, i.e.

- immunoistochemical: Ki-67 antigen for proliferative index, p-53 antigen for the expression of p-53 gene;
- hystopathological: hematosilina-eosin staining for the assessment of number of mitosis;
- ultrastructural: electron microscopy;
- nucleic acid hybridisation: Tunel method for apoptosis evaluation.

In addition, the following organs were extracted from each animal for histologic examination to assess the treatment toxicity: brain, heart, kidneys, liver, lungs, axillary and inguinal limphonodes, mediastinal limphonodes, ovaries, skin, spleen, bone marrow, subcutaneous tissue (site of tumoral cell line implantation) as well as blood tests.

The obtained results are reported in Table 3 for the first experiment and in Table 4 for the second.

TABLE 3

exposure conditions	1	2	3	4	shame-exposed
exposure duration (min)	70	70	70	70	-
time averaged field intensity (Static + ELF rms) in mT	3	3	4	6	-
field variation in mT (min-max) Static; [min-max] ELF	(4-6) [2-2]	(1.5-4) [1-1]	(2-5) [1.5-3.5]	(2-5) [1.5-3.5]	-
constant field time duration (min-max) in minutes	(5-15)	(5-20)	(5-15)	(5-20)	-
time % with co-presence of Static and ELF fields	0%	50%	50%	100%	-
S/ELF ratio (min-max)	-	(0,5-5)	(0,5-5)	(0,5-5)	-
time % with Static field alone	50%	50%	50%	0%	-
number of mice	6	6	6	6	12
extracted tumor mass volume (mm ³)	1323 ± 304	1450 ± 288	920 ± 540	650 ± 205	1492 ± 559
extract tumor mass weight (g)	1.54 ± 0.22	1.6 ± 0.39	0.98 ± 0.56	0.96 ± 0.25	1.6 ± 0.5
number of apoptotic cells per 10 HPF	98 ± 23	115 ± 20	129 ± 25	129 ± 26	40 ± 17
p53 expression per 10 HPF	35.1 ± 0.11	43.8 ± 0.16	38.2 ± 0.06	28.7 ± 0.14	73.2 ± 0.14

TABLE 4

exposure conditions	4 (see tab. 3)	shame exposed
number of mice	12	12
extracted tumor mass volume	1139 ± 509 cm ³	1914 ± 793 cm ³
extracted tumor mass weight	1.4 ± 0.7 g	2.1 ± 0.6 g
apoptosis (assessed in 50% of mice only)	72.5 ± 9.3	37.0 ± 7.4
p53	35.6 ± 6.7	78.1 ± 16.7
proliferative index	0.34 ± 0.08	0.45 ± 0.07
mitosis	24.1 ± 10.9	47.7 ± 10.1

The data reported in tables 3 and 4 show that SELF fields have an inhibitory tumor growth effect in vivo. This effect, found in both experiments, was statistically highly significant (in the first experiment, mostly for the exposure condition 4) at the Dunnet and t Student tests respectively.

At the histologic examination of 12 organs for each animal for all groups no differences were found between exposed and shame-exposed mice. No differences were also found in the blood tests. These findings prove the absence of toxicity related to the SELF fields treatment.

The ultrastructural analysis by electron microscope showed in the tumor cells of exposed animals many cellular alterations: presence of apoptotic bodies and condensed chromatin near the nuclear membrane characteristic of apoptotic events.

In addition a consistent result is represented by morphological modifications, increase of number and dimensions of mitochondria as well as number of nucleoli, presence of many vacuoles inside the cytoplasm. Non neoplastic cells (i.e. epithelial and stromal cells) showed no differences between exposed and shame-exposed animals in agreement with the absence of toxicity found in 12 normal organs examined in each animal.

The increment in apoptosis as well as the decrement in p53 gene expression found in exposed mice tumors (see tables 3 and 4) are statistically highly significant (t Student test)

Results reported in Table 3 and 4 are in agreement with those obtained in vitro and shown in Tables 1 and 2.

The effect induced by the SELF magnetic fields on p53 expression enforces the apoptosis results and is in agreement with the hypothesised biophysical mechanism (i.e. free radical recombination) by which the SELF fields have an anti-tumor effect through formation of reactive

oxygen species and the degradation of mitochondrial components.

EXAMPLE 4

In this experiment nude mice (nu/nu) previously subcutaneous inoculated with 10 million human colon adenocarcinoma cells (WiDr) were exposed to study the animal survival.

After the cell inoculation 2 groups of mice were randomly formed respectively of 16 animals exposed and 17 shame-exposed. The mice of the former group were exposed 70 minutes once a day, for 5 days a week, for their entire life beginning after 24 hours after the tumor inoculation.

The exposure conditions were the same of the experiment the results which are reported in Table 4.

As in the previous example, the mice were maintained under specific pathogen free condition supplied with "ad libitum" diet. All the tests were conducted in accordance with protocol issued by N.I.H. and N.C.I.

The antitumor effectiveness of the treatment was evaluated by using the N.C.I. formula: ratio between exposed and shame-exposed animals of the average animal life span. This average was evaluated summing for each experimental group the time of survival divided by the number of animals. The effectiveness is obtained when the N.C.I. formula gives as result an index equal or greater than 1.25.

Table 5 reports for each experimental group, the number of living animals at different times (days) from the beginning of experiment.

TABLE 5

living mice exposed/ shame-exp. (days)	16/16 (48)	16/15 (73)	15/14 (76)	14/14 (84)	13/14 (87)	12/14 (88)
living mice exposed/ shame-exp. (days)	12/13 (97)	12/12 (107)	10/12 (109)	10/10 (114)	10/9 (115)	9/8 (125)
living mice exposed/	9/7	8/6	8/5	8/4	7/4	7/3

shame-exp. (days)	(149)	(153)	(155)	(157)	(163)	(173)
living mice exposed/ shame-exp. (days)	6/3 (183)	6/2 (192)	6/0 (194)	5/0 (195)	4/0 (203)	3/0 (257)
living mice exposed/ shame-exp. (days)	2/0 (276)	1/0 (323)	0*/0 *sacrificed (326)			

The N.C.I. formula applied to the results reported in Table 5 gives an index equal to 1.31, that is greater than 1.25 . After 194 days 6 exposed mice were alive whereas all shame exposed mice were dead.

The foregoing description of specific embodiments will so fully reveal the invention according to the conceptual point of view, so that others, by applying current knowledge, will be able to modify and/or adapt for various applications such embodiments without further research and without departing from the invention, and it is therefore to be understood that such adaptations and modifications will have to be considered as equivalent to the specific embodiments. The means and the materials to realise the different functions described herein could have a different nature without, for this reason, departing from the field of the invention. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation.

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MAIN REQUESTCLAIMS

1. Apparatus for selectively interfering with pathological
cells survival processes in vitro and in vivo
5 comprising:

- means for generating static magnetic (S) fields
crossing a working environment,

- means for generating electromagnetic extremely low
frequency (ELF) fields over said working environment in
10 addition to said S fields;

characterised in that it further comprises:

- means for modulating said S fields associated to said
means for generating S fields, said means for modulating
said S fields setting the intensity of said S fields
15 between 1 and 100 mT according to a predetermined function
of intensity versus time;

- means for modulating said ELF fields associated to said
means for generating ELF fields, said means for modulating
said ELF fields setting said ELF fields according to a
20 predetermined function of amplitude of intensity between 1
and 100 mT and frequency between 1 and 1000 Hz versus
time.

2. Apparatus for selectively interfering with pathological
cells survival processes in vitro and in vivo
25 comprising:

- means for generating static magnetic (S) fields
crossing a working environment,

characterised in that it further comprises

- means for modulating said S fields associated to said
generating means, said means for modulating the S fields
30 setting the intensity of said S fields between 1 and 100
mT according to a predetermined function of intensity
versus time.

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3. Apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo

characterised in that it further comprises

- 5 - means for generating electromagnetic extremely low
frequency (ELF) fields over said working environment;
- means for modulating said ELF fields associated to said
- ~~means for generating,~~ said means for modulating said ELF
fields setting said ELF fields according to a predetermined
function of amplitude of intensity between 1 and 100 mT and
10 frequency between 1 and 1000 Hz versus time.

4. Apparatus according to any of claims 1 or 2 wherein said means for modulating said S fields comprises program means that set said intensity following a plurality of predetermined step values I_{s1} , I_{s2} , ..., I_{sn} for corresponding time intervals T_1 , T_2 , ..., T_n .

5. Apparatus according to any of claims 1 or 3 wherein said means for modulating said ELF fields comprises program means that set said intensity amplitude following a plurality of predetermined step values I_{ELF1} , I_{ELF2} , ..., I_{ELFm} for corresponding time intervals T_1 , T_2 , ..., T_m .

6. Apparatus according to any of claims 1 or 3 wherein said means for modulating said ELF fields comprises program means that set said frequency following a plurality of predetermined step values f_1, f_2, \dots, f_n , for corresponding time intervals T_1, T_2, \dots, T_n , said step values being comprised between 10 and 100 Hz.

7. Apparatus according to claim 1, wherein said means for modulating said S and ELF fields comprises program means that set an S/ELF ratio according to a plurality of predetermined step values I_{S1}/I_{ELF1} , I_{S2}/I_{ELF2} , ..., I_{Sn}/I_{ELFn} , for corresponding time intervals T_1 , T_2 , ..., T_n .

8. Apparatus according to claim 7, wherein said program

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means set said S and ELF fields according to an overall intensity between 1 and 30 mT and respectively a ratio S/ELF comprised between 0,1 and 10.

9. Apparatus according to claim 7, wherein said program
5 means set said S and ELF fields according to an overall intensity between 1 and 10 mT and respectively a ratio S/ELF comprised between 0,5 and 5.

10. Apparatus according to claims 4 to 9 wherein said
10 program means set said time intervals between 1 and 40 minutes.

11. Apparatus according to the previous claims wherein at least a portion of said working environment is defined by walls permeable to said fields.

12. Apparatus according to the previous claims, wherein
15 said means for generating said S and/or ELF fields comprise at least a first and a second coil respectively surrounding at least a portion of said working environment, said means for modulating providing to said coils DC and/or AC current
20 respectively.

13. Apparatus according to the claims from 1 to 11, wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil
25 coaxial to each other, said working environment being placed between said first and a second coil and said means for modulating providing to said coils DC and/or AC current respectively.

14. Apparatus according to the previous claims, wherein
30 means are provided for creating through said working environment a static electric field, or a low frequency variable electric field up to 1000 Hz, having intensity up to 20 kV/m.

15. The use of SELF non thermal fields for selectively

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interfering with pathological cells survival, such as in particular cells affected by cancer, viral infections, autoimmune diseases, neurodegenerative disorders, AIDS, etc., characterised in that said SELF non thermal fields have intensity comprised between 1 and 100 mT, said SELF fields being different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz.

16. The use of SELF non thermal fields for biotechnological genes modifications, such as in particular for modification of mutant p53 gene, characterised in that said SELF non thermal fields have intensity comprised between 1 and 100 mT, said SELF fields being different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz.

17. The use of SELF non thermal fields according to claims 15 or 16, wherein chemical substances are used in addition to the SELF fields.

18. The use of SELF non thermal fields according to claims 15 or 16, wherein said different sequences of S and/or ELF fields sequences are set for time intervals T_1 , T_2 , ..., T_n , and wherein in said time intervals the intensity of said S and/or ELF fields are set at steady values I_{S1} , I_{S2} , ..., I_{Sn} ; I_{ELF1} , I_{ELF2} , ..., I_{ELFn} , I_{S1}/I_{ELF1} , I_{S2}/I_{ELF2} , ..., I_{Sn}/I_{ELFn} , respectively.

19. The use of SELF non thermal fields according to claims

20. The use of SELF non thermal fields according to claims

15 or 16, wherein said S and ELF fields are set at an overall intensity between 1 and 10 mT with respectively a ratio S/ELF comprised between 0,5 and 2,5.

[illegible]

TITLE

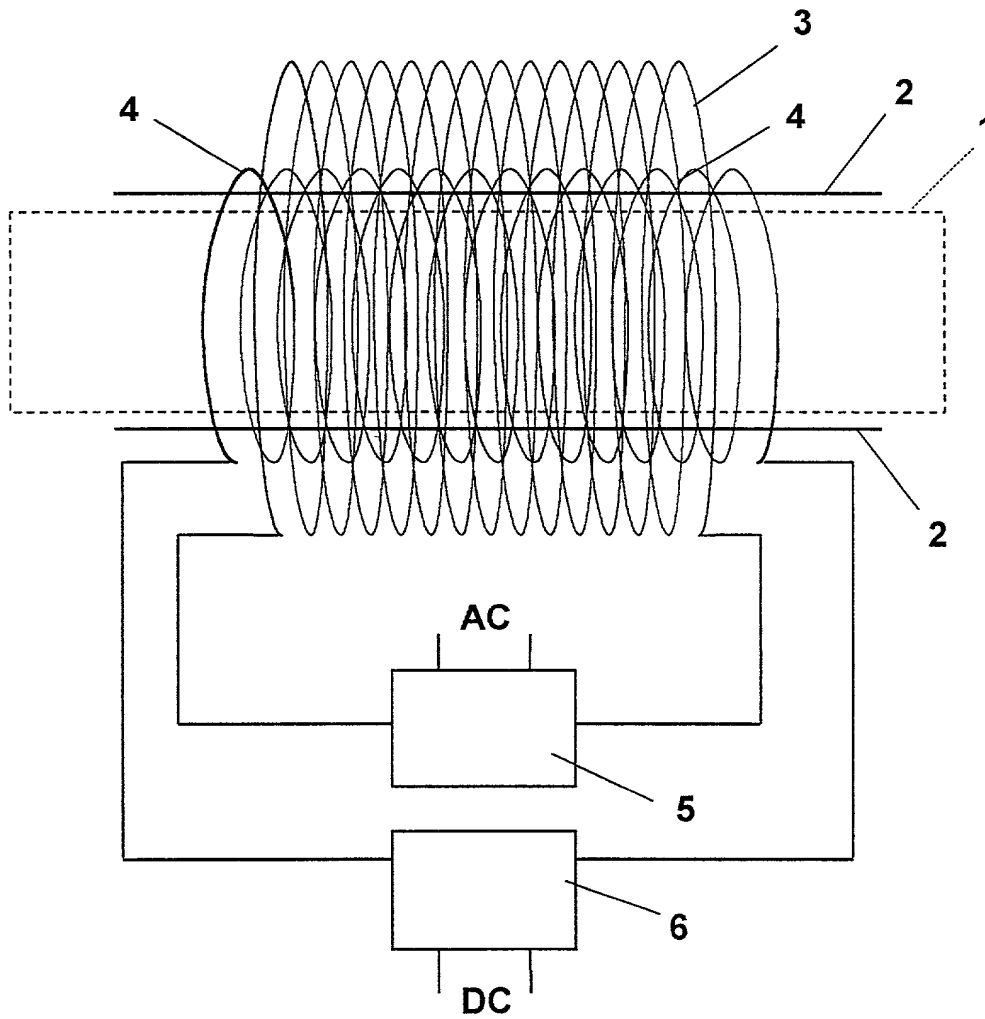
APPARATUS AND METHOD FOR INTERFERING WITH PATHOLOGICAL
CELLS SURVIVAL PROCESSES

ABSTRACT

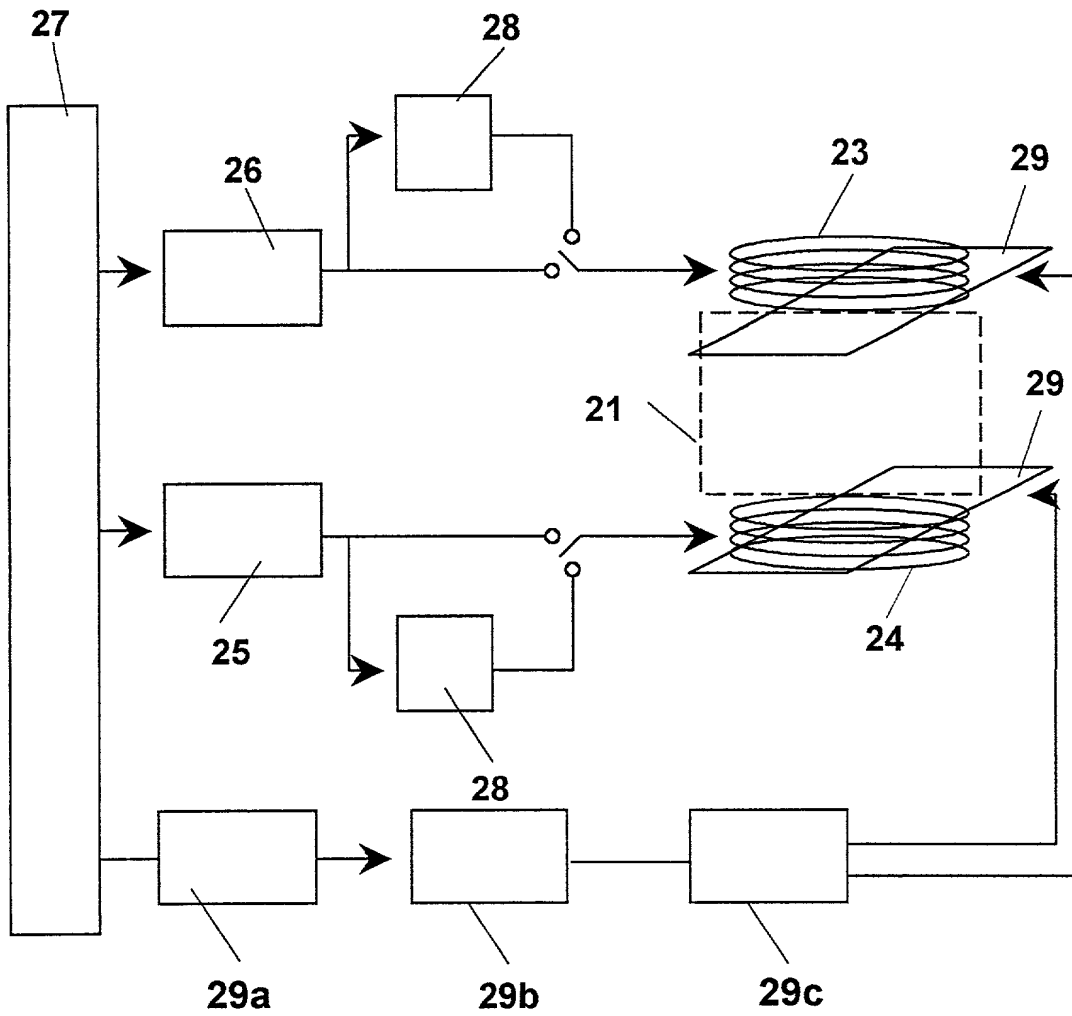
5 A method and an apparatus for interfering with
pathological cells survival processes, i.e. inducing
directly or indirectly apoptosis, on living pathological
cells, by using magnetic fields without adversely affecting
normal cells. Static (S) and extremely low frequency (ELF)
10 magnetic fields are used having low intensity comprised
between 1 and 100 mT, preferably comprised between 1 and 30
mT. In particular SELF fields are used which are different
sequences of S and/or ELF fields, i.e. S fields followed by
ELF fields, ELF fields followed by S fields, S and ELF field
15 together, as well as the presence of S or ELF fields alone,
said ELF fields having a field frequency comprised between 1
and 1000 Hz. An apparatus for carrying out the method
comprises means for generating static magnetic (S) fields
crossing a working environment and/or means for generating
20 electromagnetic extremely low frequency (ELF) fields over
the working environment in addition to the S fields. Means
are provided for modulating the S fields associated to the S
fields generating means and varying the intensity of the S
fields from 1 to 100 mT, preferably between 1 to 30 mT
25 according to a predetermined function. Means may also be
provided for modulating the ELF fields associated to the ELF
fields generating means and imposing to the ELF fields a
frequency between 1 and 1000 Hz with intensity comprised
between 1 to 100 mT, preferably between 1 and 30 mT
30 according to a predetermined function.

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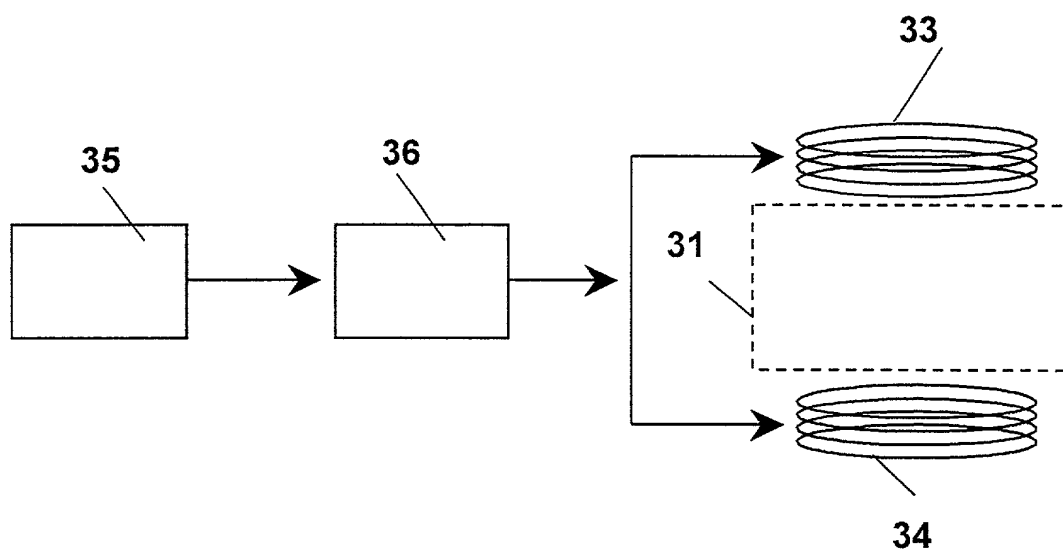
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Fig. 1

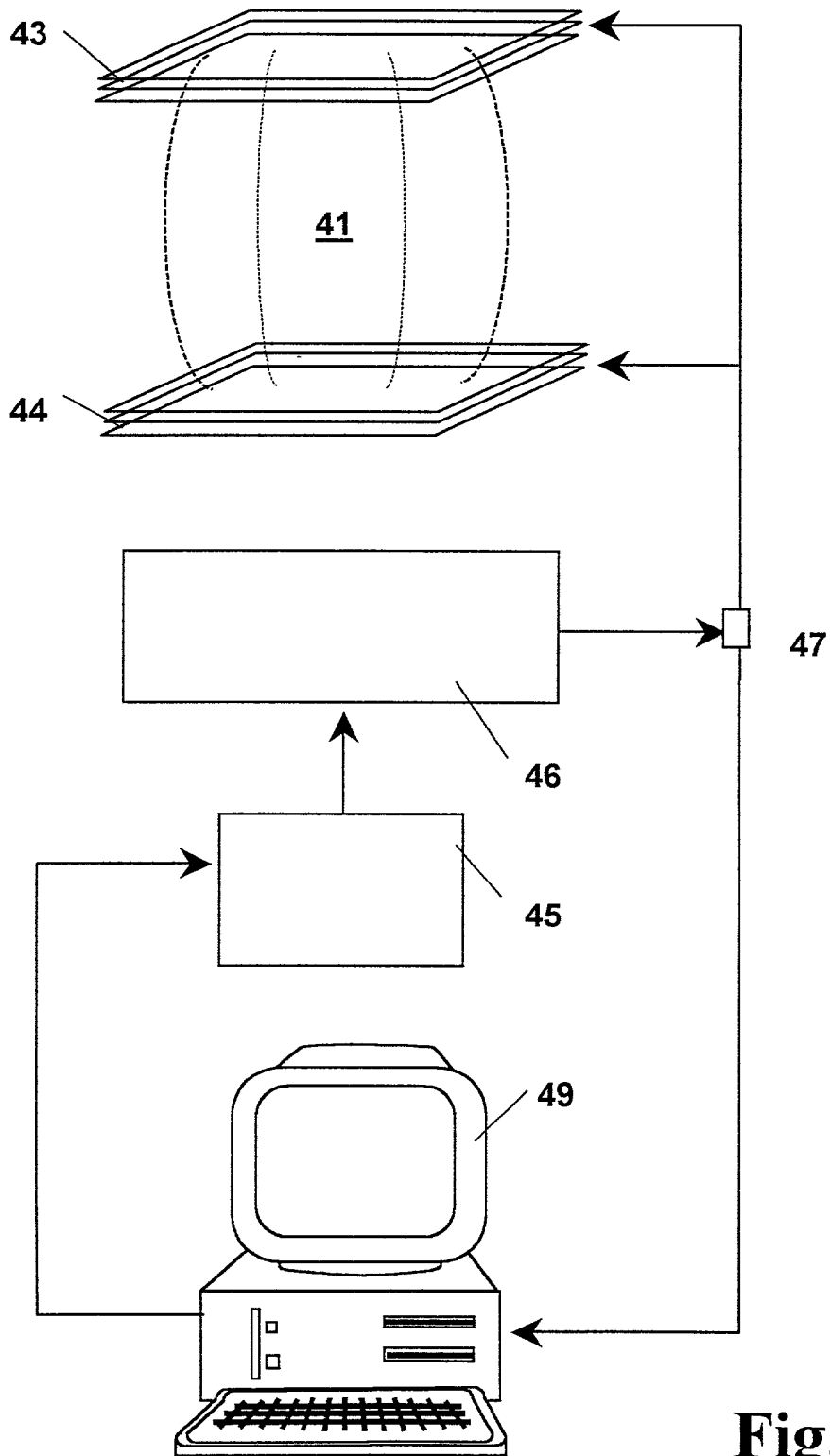
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Fig. 2

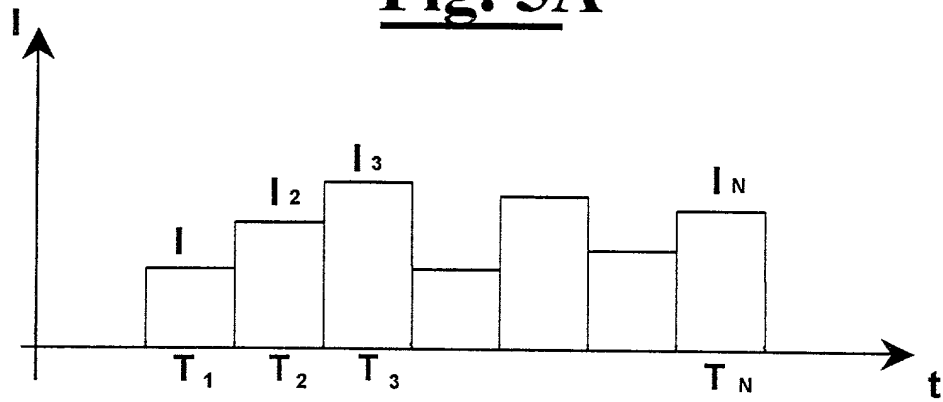
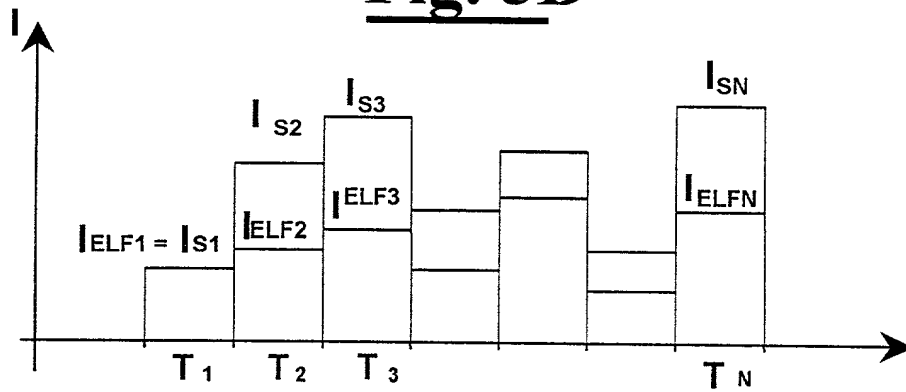
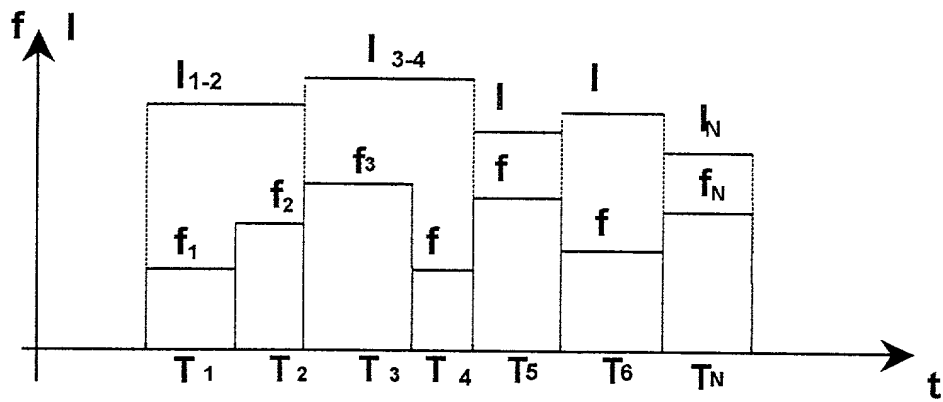
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Fig. 3

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**Fig. 4**

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Fig. 5AFig. 5BFig. 5C

Italian Language Declaration

Il sottoscritto rivendica con la presente la priorità prevista dal Titolo 35, Codice degli Stati Uniti, § 119(e)-(d) o § 365(b) in relazione a qualsiasi domanda o domande estere di brevetto o certificato di inventore, o dal Titolo 35, § 365(a) degli stessi Codici in relazione a qualsiasi domanda internazionale PCT nella quale è designato almeno un paese diverso dagli Stati Uniti, i suddetti domande e certificati essendo elencati sotto, e, spuntando le seguenti caselle, ha anche identificato sotto qualsiasi domanda estera di brevetto o certificato di inventore, o domanda internazionale PCT, la cui data di deposito preceda quella della domanda per la quale è rivendicata la priorità.

Prior Foreign Application(s)
Domande Estere Anteriori

EP93830381.4 European Application
(Number) (Country)
(Numero) (Nazione)

(Number) (Country)
(Numero) (Nazione)

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Priority Not Claimed
Diritto di priorità non rivendicato

24 June 1995 ☐
(Day/Month/Year Filed)
(Giorno/Mese/Anno di deposito)

(Day/Month/Year Filed) ☐
(Giorno/Mese/Anno di deposito)

Il sottoscritto rivendica con la presente i benefici previsti dal Titolo 35, Codici degli Stati Uniti, § 119(e), in relazione a qualsiasi domanda o domande provvisorie degli Stati Uniti elencate sotto.

Application No.) (Filing Date)
(N° della domanda) (Data di deposito)

Application No.) (Filing Date)
(N° della domanda) (Data di deposito)

Il sottoscritto rivendica con la presente i benefici previsti dal Titolo 35, Codice degli Stati Uniti, § 120, in relazione a qualsiasi domanda o domande statunitensi, o dal Titolo 35, § 365(c) degli stessi Codici in relazione a qualsiasi domanda internazionale PCT nella quale sono designati gli Stati Uniti, i suddette domande essendo elencate sotto e, nella misura in cui l'oggetto di ciascuna rivendicazione di questa domanda non sia stato esposto nella domanda statunitense o internazionale PCT anteriore nel modo previsto dal primo paragrafo del Titolo 35, Codice degli Stati Uniti, § 112, riconosce l'obbligo di rivelare informazioni essenziali ai fini della determinazione della brevettabilità ai sensi del Titolo 37, Codici dei Regolamenti Federali, § 1.56, le quali diventino disponibili durante il periodo compreso tra la data di deposito della domanda anteriore e la data di deposito nazionale o internazionale PCT della presente domanda.

PCT/EP99/04385 23 June 1999
(Application No.) (Filing Date)
(N° della domanda) (Data di deposito)

(Application No.) (Filing Date)
(N° della domanda) (Data di deposito)

Con la presente, il sottoscritto dichiara veritiere tutte le affermazioni contenute in questa domanda in relazione alle proprie conoscenze e di ritenere vere tutte le affermazioni o informazioni presentate. Dichiara inoltre che tali asserzioni sono state espresse nella piena consapevolezza che le dichiarazioni intenzionalmente false sono punibili con una multa, l'incarcerazione o entrambe, ai sensi della Sezione 1001 del Titolo 18 del Codice degli Stati Uniti e che tali dichiarazioni intenzionalmente false possono mettere a repentaglio la validità della domanda o di qualsiasi brevetto rilasciato in merito.

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

pending
(Status) (patented, pending, abandoned)
(Stato) (concessione di brevetto, in corso di esame, abbandono)

(Status) (patented, pending, abandoned)
(Stato) (concessione di brevetto, in corso di esame, abbandono)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Declaration and Power of Attorney for Patent Application**Dichiarazione e procura ai fini della domanda di brevetto****Italian Language Declaration**

Il sottoscritto inventore dichiara che:

As a below named inventor, I hereby declare that:

La propria residenza, recapito postale e cittadinanza corrispondono a quanto indicato in calce, sotto la propria firma.

My residence, post office address and citizenship are as stated next to my name.

Ritiene di essere il primo ed unico inventore originale (se viene elencato in calce un solo nominativo) o il coinventore primo ed originale (se è elencato più di un nominativo) del oggetto rivendicato e per il quale il sottoscritto presenta domanda di brevetto. La invenzione in questione è chiamata

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**APPARATUS AND METHOD FOR INTERFERING WITH
PATHOLOGICAL CELLS SURVIVAL PROCESSES**

e la sua descrizione è allegata alla presente Dichiarazione a meno che non sia spuntata la seguente casella:

the specification of which is attached hereto unless the following box is checked:

☐ Il _____
è stata depositata una domanda di brevetto
statunitense numero o una domanda di brevetto
internazionale PCT numero _____
che è stata modificata il _____
(se applicabile).

☐ was filed on _____
as United States Application Number or PCT
International Application Number _____
and was amended on _____
(if applicable).

Il sottoscritto dichiara in oltre di aver letto e compreso il contenuto della descrizione identificata in precedenza, rivendicazioni comprese, come modificati dall'eventuale modifica summenzionata.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

Il sottoscritto riconosce l'obbligo di rivelare informazioni essenziali ai fini della determinazione della brevettabilità ai sensi del Titolo 37, Codice dei Regolamenti Federali, § 1.56.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

Italian Language Declaration

PROCURA: Il sottoscritto inventore nomina con la presente il seguente avvocato o avvocati e/o agente o agenti al fine di istruire questa pratica e di condurre tutte le operazioni ad essa pertinenti presso l'Ufficio dei Brevetti e Marchi di Fabbrica: *(Elencare il nome ed il numero di matricola)*

Inviare le corrispondenza a:

Robert P. Simpson, Esq., Registration No. 33,034
George L. Snyder, Esq., Registration No. 37,729
R. Craig Kauffman, Esq., Registration No. 20,362
with the law firm of:
Simpson, Simpson & Snyder, LLP

Telefonare a: *(nome e numero telefonico)*

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*.

Send Correspondence to:

Robert P. Simpson, Esq.
Simpson, Simpson & Snyder.
5555 Main Street
Williamsville, NY 14221

Direct Telephone Calls to: *(name and telephone number)*

Robert P. Simpson, Esq., Telephone No. 716-626-1654

Nome e cognome dell'unico o del primo inventore 20/12/00	Full name of sole or first inventor TOFANI Santi
Firma dell'inventore _____ Data _____	Inventor's signature _____ Date _____
Residenza _____	Residence Via Bruetto, 18 I-10010 <u>BUROLO (TO)</u> ITALY <u>ITX</u>
Cittadinanza _____	Citizenship Italian
Recapito postale _____	Post Office Address Same as above
Nome e cognome dell'eventuale secondo coinventore _____	Full name of second joint inventor, if any
Firma del secondo coinventore _____ Data _____	Second Inventor's signature _____ Date _____
Residenza _____	Residence
Cittadinanza _____	Citizenship
Recapito postale _____	Post Office Address

(Fornire le stesse informazioni e le firme del terzo e degli ulteriori coinventori.)

(Supply similar information and signature for third and subsequent joint inventors.)